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Cell cycle inhibitors in T cell tolerance and autoimmunity control

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INHIBIDORES DEL CICLO CELULAR EN LA TOLERANCIA DE CÉLULAS T Y EN EL CONTROL DE LA AUTOINMUNIDAD

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RESUMEN

Durante los últimos años, se ha acumulado evidencia que demuestra que la regulación del ciclo celular de las células es esencial para establecer tolerancia y suprimir autoinmunidad. Aunque la apoptosis se ha considerado un mecanismo importante en el control del desarrollo de tolerancia y autoinmunidad, la regulación del ciclo celular también constituye una vía alternativa en la prevención de la autoreactividad. Diferentes moléculas asociadas al ciclo celular actúan como supresoras de la autoinmunidad. Se ha demostrado recientemente que los inhibidores del ciclo celular p21 y p27 controlan la tolerancia de las células T, mientras que p21 también limita el desarrollo de la autoinmunidad. En esta revisión exploraremos los efectos de p21 y p27 en la inducción de la tolerancia de las células T y discutiremos la asociación entre pérdida de tolerancia y desarrollo de autoinmunidad en ratones $p21^{-/-}$.

PALABRAS CLAVE: Ciclo celular/ p21/ p27/ Lupus/ Autoinmunidad/ Células T.

ABSTRACT

Over the last few years, evidence has accumulated showing that cell cycle regulation of T cells is essential to establish tolerance and to suppress autoimmunity. Although apoptosis has been considered an important mechanism in the control of tolerance and autoimmunity development, cell cycle regulation also constitutes a pivotal alternative pathway in the prevention of autoreactivity. Several cell cycle-associated molecules act as autoimmunity suppressors. The cell cycle inhibitors p21 and p27 have recently been shown to control T cell tolerance, while p21 also restrains development of autoimmunity. In this review, we will explore the effects of p21 and p27 in T cell tolerance induction and discuss the association between tolerance loss and autoimmunity development in $p21^{-/-}$ mice.

KEY WORDS: Cell cycle/ p21/ p27/ Lupus/ Autoimmunity/ T cells.

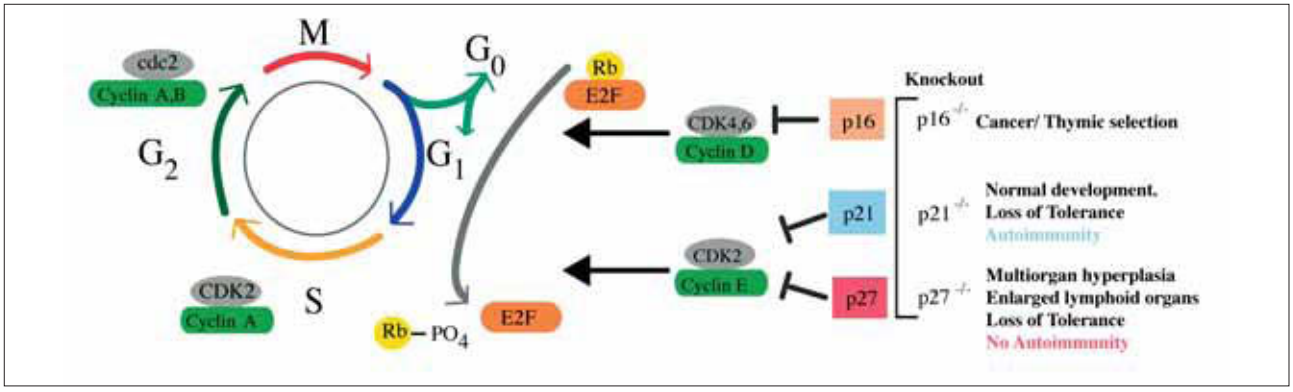

Figure 1. *Immunological effects of cell cycle regulation. The figure shows the four cell cycle phases, the cyclin-Cdk interactions and the cell cycle inhibition points of p16, p21 and p27, as well as their involvement in immunity.*

INTRODUCTION

Immune cell proliferation is mandatory for the immune system development and function. In bone marrow and thymus, immune cell precursors cycle vigorously, to expand and differentiate into the diverse constituents of the immune system. T cells represent fundamental components of the immune response and are required to combat microbial infections. They proliferate extensively in the thymus, where they differentiate into mature $CD4^+$ or $CD8^+$ cells and are exported to the periphery.

Following microbial invasion, mature T lymphocytes are exposed to antigen, become activated, and undergo intense, prolonged and repeated proliferation to establish a rapid immune response and generate immunological memory. This expansion is destined to contract, however, to maintain the T cell population in a stable cellular proportion within the immune system. This condition is known as homeostasis, in which total lymphocyte number, the proportion of naïve and memory T cells, and the extent of T cell memory expansion are regulated. The basis of homeostasis is the maintenance of a balance between proliferation/expansion and death of immune cells[1].

Ever since the discovery of apoptosis in immune system cells, and particularly in T cells, programmed cell death or apoptosis of stimulated cells has been considered the major pathway for homeostasis and tolerance control. Nevertheless, recent findings, including data from our laboratory, provide evidence that T cell tolerance and homeostasis are two processes that are highly dependent on cell cycle regulation in activated/memory T cells. Thus, cell cycle regulation controls both T cell tolerance and homeostasis, and aberrant proliferation is associated with autoimmunity development[2-6]. This review will focus mainly on the role of the cell cycle inhibitors p21 and p27 in tolerance and homeostasis, as they are well-studied molecules in the context of T cell function.

THE CELL CYCLE MACHINERY AND ITS ASSOCIATION WITH T CELL FUNCTION

Division is a complex process and its control is fundamental for all cell types. Cell cycle progression depends on intra- and extracellular signals that affect the cell cycle machinery and are translated into proliferation, cell cycle arrest, or differentiation.

In general, cell cycle progression takes place in four phases after stimulation of quiescent G0 cells: G1, the first gap phase; S, the DNA replication phase; G2, the second gap phase and M, mitosis, in which genetic information is distributed between two daughter cells. Cell cycle progression is dependent on cyclins, which associate with catalytically active cyclin-dependent kinases (Cdk) to form active complexes. Cdk inhibitors act as negative regulators of these complexes to control cell cycle progression. This progression is characterized by cyclin expression, Cdk activation, and phosphorylation of appropriate substrates. A key substrate for cell cycle activation is the retinoblastoma (Rb) gene. Phosphorylation of Rb uncouples its binding to E2F-type transcription factors, resulting in activation of S phase gene transcription[7-9].

We present a scheme that depicts the classical sequence of events required for cell cycle progression (Fig. 1). During G1/S phase progression, cyclins D (D1–D3) act in mid-G1, followed by cyclin E and cyclin A at the G1/S boundary; cyclins A and B operate during S and G2/M phases. The Cdk require association with cyclins as well as phosphorylation for activity. Cdk4 and Cdk6 are associated with cyclins D, whereas cyclins A and E assemble with Cdk2. Nevertheless, Cdk2 may not be needed for progression to mitosis, since Cdk2 deficiency is not lethal in mice[10] and Cdk1 (also known as cdc2) can replace Cdk2 function[11]. The activity of cyclin:Cdk complexes is repressed by Cdk inhibitors, which constrain entry into S phase. Two classes of inhibitors have been defined, on the basis of their structural characteristics and Cdk targets. p15, p16, p18 and p19 are defined as INK4 (inhibitors of Cdk4),

and associate solely with Cdk4 and Cdk6. The other inhibitor group includes p21, p27 and p57 (the Cip/Kip family), which interfere with cycling by binding to both cyclin and Cdk subunits and inhibit all Cdk involved in G1/S transition[12].

p16 deficiency can lead to an overall increase in lymphoid organ size, affects thymic selection, but is not linked to loss of tolerance (Fig. 1)[13]. Genetic deletion of p27 leads to multi-organ hyperplasia and enlargement of lymphoid organs[14-16]. Recent findings point to p27 as a molecule involved in peripheral tolerance[17, 18]. On the other hand, $p21^{-/-}$ mice develop normally, progressively losing tolerance to DNA and displaying severe autoimmunity with age[3, 4]. Loss of tolerance is associated with a cyclin-Cdk complex that is targeted by both p21 and p27 (Fig. 1); it nonetheless appears that p21 is an immune system-specific cell cycle inhibitor, whereas p27, in addition to its role in immunity, is a broad cell cycle regulator.

APOPTOSIS *VERSUS* CELL CYCLE REGULATION IN T CELL TOLERANCE AND HOMEOSTASIS

Elimination of expanded or autoreactive T cells apparently leads to homeostasis and tolerance. It is therefore generally accepted that apoptosis could control these two processes, and that defective apoptosis could lead to the break of homeostasis and tolerance. This view is based on the phenotype of Fas-deficient *(lpr)* mice. Fas is the prototype apoptosis-inducing molecule, and is essential for T cell homeostasis and tolerance induction[19]. Interaction of Fas with its ligand induces apoptosis by recruiting an adaptor protein, the Fas-associated death domain (FADD), which activates caspase-8 and initiates the apoptosis cascade[20].

The *lpr* mice develop lymphadenopathy (with increased $CD4^{-}CD8^{-}TCR^{+}$ T cells; double-negative, DN), although the basis of lymphadenopathy development remains elusive[20, 21]. The first evidence that defective apoptosis alone cannot account for T cell accumulation derived from the analysis of transgenic mice overexpressing the caspase-8 inhibitor CrmA in T cells[22, 23]. After secondary stimulation, apoptosis was inhibited *in vitro*, but DN T cell generation and lymphadenopathy *in vivo* were unaffected. This suggested that, in addition to its role in apoptosis, Fas might have another function in homeostasis control[22, 23].

Analysis of mouse models in which FADD was deactivated or deleted in T cells showed that this adaptor protein is needed for primary T cell proliferation[23-25]. Caspases were also reported to be necessary for primary T cell responses[26-28]. Nevertheless, deletion of FADD or caspase-8 did not reproduce the *lpr* phenotype, leading to a speculation that there is an association between Fas-driven apoptosis and T cell proliferation[24, 25, 27, 29]. The *in vivo lpr* phenotype was also thought to be the consequence of the proliferative and/or apoptotic defects[30]. This view is supported by results showing that *lpr* mice and autoimmune lymphoproliferative syndrome (ALPS) patients with mutations in the Fas death domain present T cell accumulation and hyperproliferation *in vivo*[31-35]. Explanation of homeostasis and tolerance loss due to a classical apoptosis defect thus requires that we consider the consequences of defective apoptosis in T cell proliferation.

Concurring with speculations that T cell tolerance and homeostasis may be conditioned by cell cycle-associated factors, we showed that a p21 deficiency-induced defect in cell cycle regulation leads to *in vivo* loss of tolerance and homeostasis[3, 4]. In addition to its cell cycle regulatory role, p21 is proposed to act as an apoptosis regulator[36]. The effect of p21 deficiency on apoptosis is debated, however; some studies report that p21 inhibits apoptosis[37, 38], whereas others indicate that p21 promotes apoptosis[39, 40]. These contrasting views might be explained by considering that the effect of p21 on apoptosis could be cell type-dependent.

For several years, apoptosis has been considered the principal defect leading to loss of tolerance and development of autoreactivity. Perhaps this led to the consideration that lack of p21 in T cells could provoke defective T cell apoptosis. BXSB is an atypical lupus model in which male but not female mice develop lupus-like disease. At 24 h after T cell receptor (TCR) stimulation, BXSB $p21^{-/-}$ T cells hyperproliferate and their apoptosis levels are similar to those of BXSB controls[37, 41]. Nevertheless, increased apoptosis is detected three days after secondary stimulation of BXSB $p21^{-/-}$ T cells[37]. In our studies, we used a variety of different systems to study apoptosis and showed that p21 deficiency does not influence T cell death[3, 4]. Nonetheless, $p21^{-/-}$ T cells that survive apoptosis 24 h after secondary stimulation proliferate excessively compared to controls, reaching maximum hyperproliferation levels three days after secondary challenge[3]. These findings raise the possibility that the increased apoptosis observed in T cells from BXSB mice three days after secondary challenge may be an indirect effect of T cell hyperproliferation. This issue must be clarified in the future, and could provide a means to analyze the relationship between apoptosis and cell cycle.

The view that the disease in BXSB mice is ameliorated due to increased apoptosis of $p21^{-/-}$ T cells has been challenged[42]; $p21^{-/-}$ T cells from BXSB males and females exhibit increased apoptosis, although in females this apoptosis does not eliminate memory T cells, which presumably provoke disease[37, 41]. Finally, the autoimmunity-prone BXSB background may confound the effects of p21 in memory T cell generation and autoimmunity. We believe that the non-autoimmune C57BL/6 background offers more clear-cut conditions for defining the role of p21 in T cell apoptosis and/or proliferation.

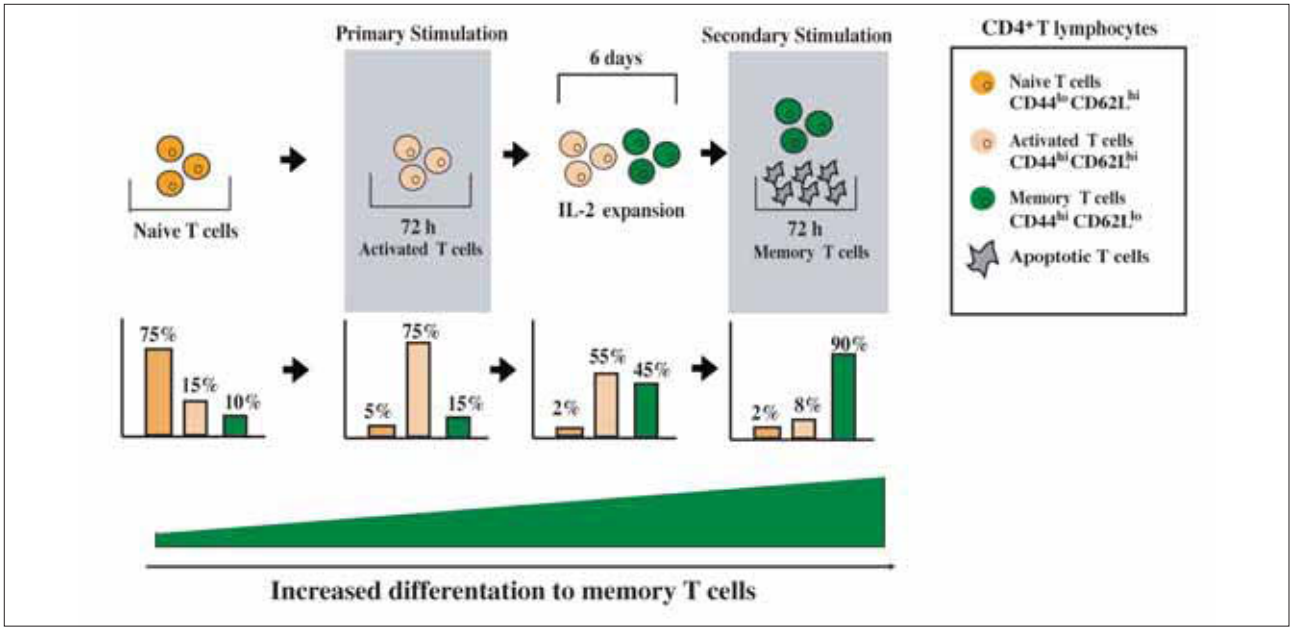

Figure 2. *Extended T cell stimulation leads to increased proportion of memory T cells. Naïve T cells were submitted to primary mitogenic stimulation with concanavalin A (ConA), expanded by IL-2 and restimulated with ConA. The memory phenotype of live T cells was monitored during the various phases of the stimulation.*

Association of T cell tolerance and cell cycle regulation has also been reported for mice deficient in other cell cycle-controlling molecules such as the p53 effector gene Gadd45a[43] and E2F2[44]. p27, another cell cycle inhibitor that is associated with primary T cell proliferation and anergy induction, was recently shown to regulate T cell tolerance in *in vivo* models[17, 18]. These data confirmed our earlier hypothesis that, in addition to alterations in apoptosis, defective cell cycle control could be determinant for loss of tolerance, homeostasis and development of autoimmunity[2].

Overall, it is clearly established that in addition to apoptosis, cell cycle regulation has a fundamental role in homeostasis and tolerance. Defining the relationship between apoptosis and the cell cycle in tolerance establishment thus appears to be a future research challenge.

CELL CYCLE REGULATION IN TOLERANCE AND AUTOREACTIVITY

Our studies of p21-deficient mice and reports on p27-deficient mice have established that p21 and p27 are both essential for *in vivo* tolerance induction. Some mechanistic insights have been offered that explain how these two Cdk inhibitors contribute to T cell tolerance.

To dissect the precise role of p21 in the immune response independently of the influence of the mixed background (129/Sv x C57BL/6) used in many studies of cell cycle-associated deficiencies, we generated and analyzed C57BL/6 $p21^{-/-}$ mice. p21 controls the proliferation of activated/memory T cells but not of naïve T cells[3]. Our studies were based on a classical model of memory T cell generation[45] in which homeostasis is presumed to occur by apoptosis (Fig. 2). After primary mitogenic stimulation, $CD4^+$ T cells were expanded with IL-2; at the end of the expansion period, half of the T cells had reached a memory phenotype (Fig. 2). Cells were then rechallenged as for the primary stimulation; while the majority of T cells died through Fas-mediated apoptosis, a proportion of memory phenotype T cells survived.

Our data from the repeated T cell activation experiments showed that p21 does not affect apoptosis[3]. Apoptosis-resistant T cells nonetheless require p21 to control their proliferation, which may comprise a homeostatic mechanism that regulates memory T cell expansion. In addition to repetitive antigen challenge, memory T cells can be derived after a single TCR stimulation[46, 47]. According to this model and as shown by our results, memory T cells are generated after extensive IL-2 treatment following primary stimulation (Figs. 2, 3). Hyperproliferation of activated/memory $p21^{-/-}$ T cells shows that memory T cells require p21 for control of proliferation (Fig. 3). As apoptosis is minimal during IL-2-dependent T cell expansion, p21 control of proliferation appears to be the major pathway that limits accumulation of activated/memory T cells. These results suggest that certain forms of memory T cell homeostasis can be apoptosis-independent and are regulated

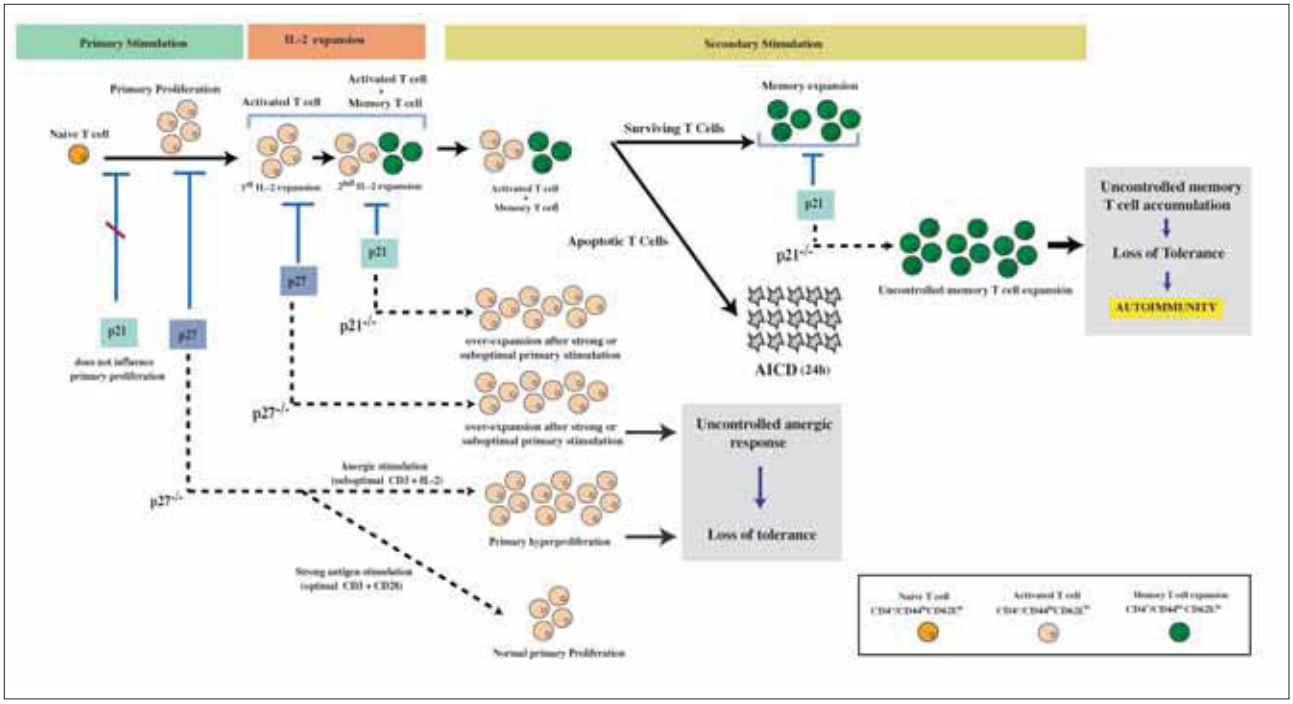

Figure 3. *A model showing proliferation control by p21 and p27 after extended stimulation. p21 control checkpoints are represented at various times, including primary stimulation, IL-2 expansion and secondary stimulation. The red bar indicates that p21 does not affect primary stimulation. p21 deficiency leads to T cell hyperproliferation, loss of tolerance, and autoimmunity. Proliferation control by p27 is indicated. p27 deficiency is translated into a deregulated response to anergy-inducing conditions and to loss of T cell tolerance.*

by cell cycle control mechanisms. The scheme shows the experimental approaches used to generate activated/memory T cells and indicates p21 regulation points of T cell proliferation, as well as immune functions, such as memory, homeostasis and tolerance, that may be controlled by p21 (Fig. 3).

We thus showed a role for p21 in the regulation of activated/memory T cell proliferation, although the exact mechanism by which p21 exerts its control remains to be defined. Our data point to the possibility that the T cell differentiation stage may influence this mechanism. Indeed, our results show a requirement for p21 in memory T cell proliferation, but not in naïve T cells (Fig. 3). Regulation of the cell cycle machinery apparently differs in the two types of T cells. It seems likely that primary stimulation of T cells leads to a memory phenotype that increases the requirement for p21 in the control of proliferation. One explanation for the distinct role of p21 in naïve and memory T cell proliferation could be based on epigenetic modifications that occur during the transition from naïve to memory T cells[48]. Epistatically modified memory T cells are thus programmed to respond rapidly after antigen encounter[48], and may require p21 to moderate this excessive response.

The association between p21 deficiency and T cell memory has *in vivo* implications, since immunization studies using ovalbumin (OVA) as antigen show that p21 deficiency confers hyperresponsiveness on OVA-specific T cells[3]. By regulating proliferation of memory T cells, p21 may thus control their homeostasis, assuring an adequate proportion of memory cells in the total T cell population. To explain anti-DNA antibody production and autoimmunity in $p21^{-/-}$ mice, we suggest that p21 deficiency may lead to memory T cell hyperproliferation and, following persistent encounter with autoantigens, to deregulated homeostasis and loss of tolerance.

Several groups have addressed the role of the cell cycle regulator p27 in primary T cell proliferation and tolerance[8]. Their main conclusion is that p27 has a significant role in anergy induction, at least for $CD4^+$ T cells. p27-deficient T cells escape anergy when activated without CD28 costimulation, or in certain other conditions[49-51]. As is the case for p21, p27 does not influence primary T proliferation following complete costimulation. It has thus been generally assumed that p27 is linked to $CD4^+$ anergy induction (Fig. 3)[52]. It has also been shown that $p27^{-/-}$ $CD4^+$ T cells hyperproliferate during the IL-2 expansion period, as do $p21^{-/-}$ $CD4^+$ T cells[3, 51]. The difference lies in that $p27^{-/-}$ T cells hyperproliferate at the beginning of the IL-2 expansion treatment, whereas $p21^{-/-}$ T cells require more extensive IL 2 exposure to manifest their hyperproliferative potential (Fig. 3). Moreover, as for the p21-

deficient mice[3], the *in vivo* CD4+ T cell response to a model antigen is also increased in the absence of p27[51, 53]. These common points between p21- and p27- deficient T cells suggest that, at least to some extent, these two Cdk2 inhibitors affect the immune response similarly.

Although mice deficient in p27 do not develop spontaneous autoimmunity, as is the case for p21$^{-/-}$ mice, it was recently established that p27$^{-/-}$ T cells are also prone to break tolerance *in vivo*[17, 18]. In one case, blockade of CD28 and CD40 costimulation, which leads to long-term allograft survival in wild-type mice, resulted in acute rejection of a MHC-mismatched cardiac allograft in p27$^{-/-}$ mice. This rejection was associated with massive lymphocyte expansion, and increased CD4+ T cell infiltration and proliferation in the cardiac grafts[18]. Another study examined p27 *in vivo* function during tolerance induction in naïve p27$^{-/-}$ TCR-transgenic T cells; these cells remained responsive after adoptive transfer into syngeneic wild-type recipient mice that was followed by a tolerizing stimulus *in vivo*[17].

As previous research indicated that p27 controls anergy induction in certain *in vitro* models, the loss of tolerance by p27$^{-/-}$ T cells has been attributed to anergy induction-associated defects[52]. Whether this loss of tolerance is due to the anergy-associated defects in T cells or simply to the hyperproliferative properties of p27$^{-/-}$ T cells has yet to be clearly defined.

In conclusion, *in vivo* tolerance induction requires p27 expression in naïve T cells or p21 expression by memory T cells. The major difference between the tolerance-inducing effects of these two cell cycle regulators is that p21 is necessary to maintain tolerance under physiological conditions, while the need for p27 is manifested when the tolerance machinery is challenged. Break of tolerance thus occurs spontaneously in p21$^{-/-}$ mice that develop anti-DNA autoantibodies and autoimmune disease, but not in p27$^{-/-}$ mice, which remain free of autoimmunity.

The unifying concept derived from the association of p21 or p27 expression in T cells and tolerance establishment is that T cell tolerance is highly dependent on cell cycle regulation after T cell stimulation. Analysis of the factors that control T cell proliferation will therefore elucidate the mechanisms of tolerance induction and maintenance.

THE ROLE OF CELL CYCLE REGULATION IN AUTOIMMUNITY DEVELOPMENT

C57BL/6 p21$^{-/-}$ mice develop all lupus characteristics and eventually develop moderate glomerulonephritis; this is more pronounced in female mice. In the case of 129/Sv x C57BL/6 p21$^{-/-}$ mice, females develop acute disease that leads to death. It appears that genetic elements of the mixed background[54] and the female hormone environment[55, 56], in conjunction with the lack of p21, control disease severity[4, 43]. This result was anticipated due to the multifactorial nature of lupus[4, 57]. Indeed, genetic background is a determining factor for lupus disease development in mice with mutations in lupus-related genes[58, 59]; thus, whereas lack of Fas leads to full-blown lupus in combination with the MRL background, it causes milder disease in C57BL/6 mice[60]. These data establish that p21 deficiency enhances autoimmunity and suggest that cell cycle deregulation constitutes an alternative-to-apoptosis pathway that leads to break of tolerance and autoimmunity development.

Deregulation of apoptosis in lymphocytes could provoke autoimmunity, since apoptosis defects lead to defective homeostasis and T cell accumulation, causing break of tolerance[1, 61]. This is the case for mice with defects in tumor necrosis factor (TNF) family apoptosis-related molecules such as the Fas/Fas signaling ligand system[62], as well as in Bcl-2-overexpressing[63] or Bim-deficient mice[64]. The idea that cell cycle deregulation can lead to autoimmune disease has recently received further support. Thus, in addition to autoimmunity development by p21$^{-/-}$ mice, mice deficient in other cell cycle-associated molecules such as the p53 effector gene Gadd45a[43] and E2F2[44] also show autoimmune manifestations, as do Gadd45β/Gadd45γ double-deficient mice[65]. These findings further strengthen our original hypothesis that, in addition to apoptotic defects, deficient cell cycle control could be a determining factor in loss of tolerance and autoimmunity development[2]. T cell proliferation anomalies are also associated to diabetes development[5, 6], suggesting a broader relationship between cell cycle and autoimmunity.

Understanding the mechanism of autoimmunity development on the basis of current studies of mice lacking cell cycle-associated molecules entails a major caveat, since all the mutant mice are on the mixed 129/Sv x C57BL/6 background. It is now well established that (129/Sv x C57BL/6) F1 or F2 mice develop mild humoral autoimmunity due to epistatic interactions between the 129/Sv and the C57BL/6 genomes[54]. The autoimmune suppressor potential of cell cycle-associated molecules thus cannot be precisely evaluated unless the deletion of these molecules is studied on a pure non-autoimmune background.

As discussed earlier, we introduced the p21 deficiency onto the autoimmunity-resistant C57BL/6 background and found that lack of p21 conferred a predisposition to autoimmunity on these mice[3]. Further support for p21 as a key autoimmunity suppressor was provided by studies showing that deletion of p21 in Gadd45a-deficient mice, which show a certain predisposition to autoimmunity, dramatically increased autoimmunity and mortality in the doubly-deficient mice[43]. p21 deletion combined with certain elements of the 129/Sv x

C57BL/6 background[4] or with Gadd45a deletion[43] can therefore induce severe autoimmunity. p21 deficiency thus emerges as an autoimmunity accelerator that is background-dependent, as are other major autoimmunity accelerators such as the *lpr* or the *Yaa* genes[66].

BXSB $p21^{-/-}$ mice have been also studied. The BXSB lupus-like disease depends on the yet uncharacterized *Yaa* gene on the X chromosome[67], and only male mice are prone to disease development. Male BXSB $p21^{-/-}$ mice were reported to present reduced autoimmune manifestations rather than increased autoimmunity[37]. The apparent improvement in male BXSB $p21^{-/-}$ mouse disease might indicate an interaction between the p21 and the *Yaa* genes, particularly at the B cell level, since it was clearly demonstrated that the direct effect of the *Yaa* gene occurs in B rather than in T cells[67, 68]. Lack of p21 could lead to reduced B cell activation via its interaction with the *Yaa* gene; indeed, in caspase-3 knockout mice, which have an overactive B cell response, p21 deletion reduces B cell hyperproliferation[69].

Analysis of p21 deletion on different murine backgrounds points to possible p21 influence not only on T cell proliferation and thus T cell homeostasis and tolerance, but also on other immune system components such as B cells or even on innate immunity. Deciphering the precise effect of p21 in the overall immune response will help to evaluate the potential of p21-related therapeutic approaches to disease. Indeed, it was recently confirmed that inhibition of the cell cycle using p21 analogues had a therapeutic effect on lupus development in (NZB X NZW)F_1 mice[42]. This finding firmly supports our results demonstrating that p21 is a potent lupus autoimmunity suppressor.

Both p21 and p27 control tolerance, as discussed above. Nevertheless, only $p21^{-/-}$ mice develop spontaneous autoimmunity. It therefore remains unclear what factors promote development of autoimmunity in $p21^{-/-}$ mice, and why $p27^{-/-}$ mice are autoimmunity-resistant. We can consider at least three possible explanations for this difference. First, $p21^{-/-}$ mice develop normally and are cancer-free to an advanced age[70, 71], while $p27^{-/-}$ mice present generalized organomegaly and predisposition to cancer[14-16]; the phenotypic abnormalities of $p27^{-/-}$ mice might disguise or suppress a putative predisposition to autoimmunity. Second, as shown in Figure 3, the hyperproliferative characteristics of $p21^{-/-}$ and $p27^{-/-}$ T cells are manifested at different times during repetitive mitogenic stimulation experiments. Whereas p27 influences T cell responses after primary stimulation, p21 clearly affects the secondary stimulation responses and is associated with the memory T cell response. This disparity may be the key to spontaneous anti-DNA autoantibody development, due to the capacity of memory $p21^{-/-}$ T cells to hyperproliferate after several rounds of autoantigen presentation. Third, as mentioned previously, lupus-like disease may depend on the effect of p21 not only on T cells but also on other immune cells. Possible differences in the p21 or p27 contribution to other-than-T cell immune system components may explain spontaneous autoimmunity development in $p21^{-/-}$ but not in $p27^{-/-}$ mice.

In conclusion, molecules classically considered to pertain to the cell cycle are pivotal for tolerance induction, maintenance of T cell homeostasis, and suppression of autoimmune disease. Deregulation of cell cycle control therefore has fundamental consequences in the immune response. Since p21 controls T cell tolerance and is an autoimmunity suppressor, it is evident that therapeutic approaches based on cell cycle inhibition can be considered for targeting autoimmune disease.

PERSPECTIVES: USE OF CELL CYCLE CONTROL IN AUTOIMMUNE DISEASE TREATMENT

The data showing that p21 and p27 control T cell tolerance suggest novel approaches for intervention in autoimmunity. Further research is needed to establish the exact pathways through which these two cell cycle inhibitors control T cell proliferation and tolerance. This could lead to the discovery of new targets for tolerance induction and to the design of therapeutic approaches for autoreactivity. Nevertheless, the possibility that cell cycle inhibition could interfere with immune responses beyond T cell function must be explored in therapeutics design. Finally, it appears that since $p21^{-/-}$ mice develop normally, treatments that target the p21 cell cycle inhibition pathway might be directed mainly to T memory cell activity, rather than producing adverse effects on the overall immune response or at a systemic level.

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DISCLOSURES

The authors declare that they have no financial conflict of interest with reference to this manuscript.

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